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Address for correspondence and reprints: Dr. Virginia P. Sybert, Division of Dermatology, H414, Children's Hospital and Medical Center, CH-25, Box 5371, Seattle, WA 98105-0371.

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### Isolated Case of Mental Retardation and Ataxia Due to a De Novo Mitochondrial T8993G Mutation

To the Editor:

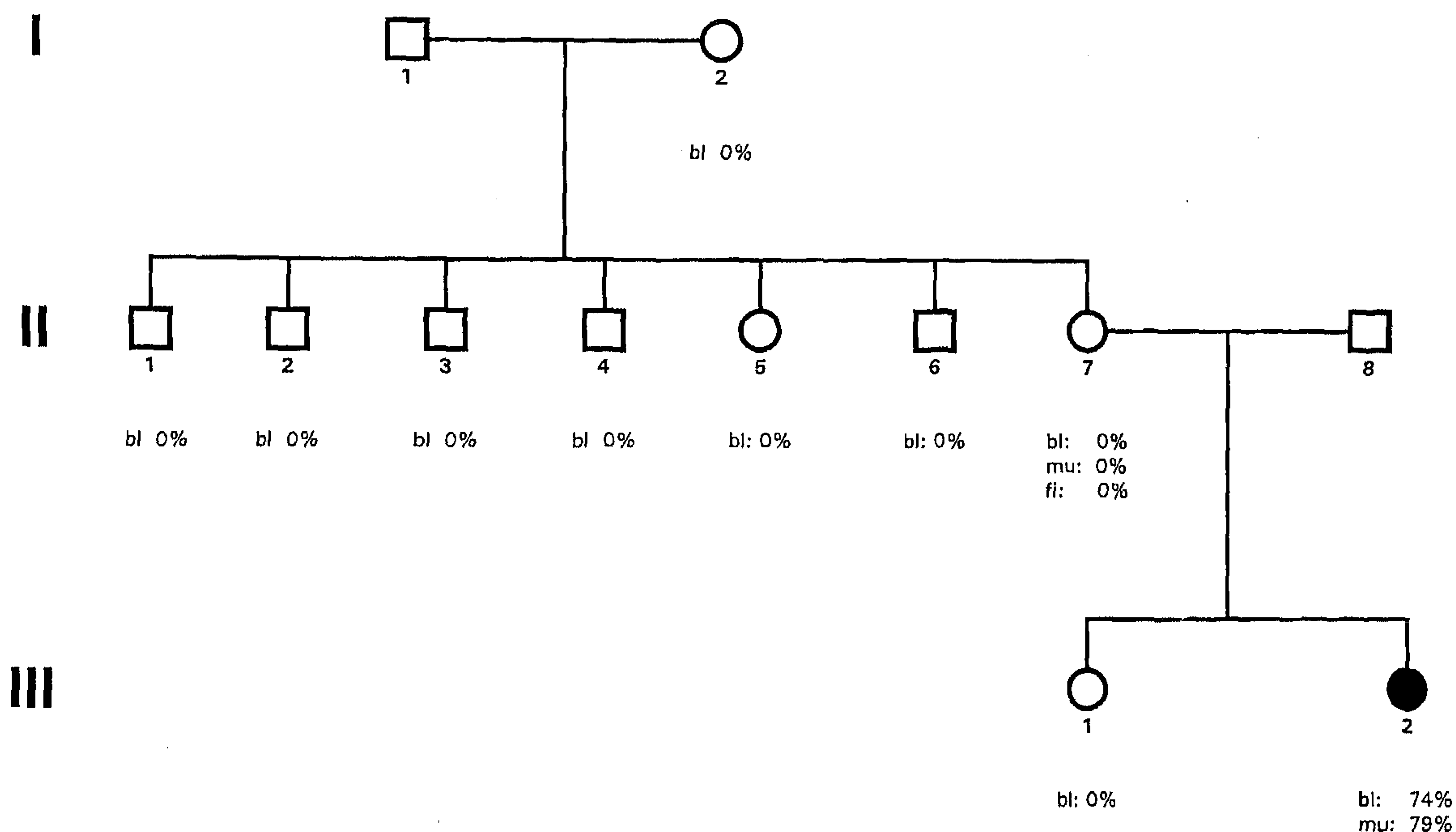
Neurogenic muscle weakness, ataxia, and retinitis pigmentosa (NARP) and subacute necrotizing encephalomyelopathy (Leigh disease) are both associated with an alteration of nt 8993 in the mitochondrial ATPase 6 gene. In NARP, the T-to-G transversion at that position changes leucine into arginine (Holt et al. 1990). In Leigh syndrome, the same mutation can be found (Tatuch et al. 1992), as can a T-to-C transition (de Vries et al. 1993), which changes this leucine into proline. Clinical manifestations occur for NARP when ~60%-90% mutated mtDNA is present. In case of Leigh, these percentages usually exceed 95% (Tatuch et al. 1994). It is known that this mutation can segregate very rapidly within pedigrees (Santorelli et al. 1993; Tulinius et al. 1995). Here we report on a sporadic case with mental retardation and ataxia without retinitis pigmentosa in which the T8993G mutation was found.

The patient was born, after an uncomplicated pregnancy, with a perinatal asphyxia. The neonatal period was unremarkable. Because of complaints about exercise intolerance and slow recovery after viral infections, she was medically investigated at the age of 5 years. Clinical examination determined her to be moderately mentally retarded. She showed a cerebellar ataxia with a normal muscle tone and extensor plantar responses. No signs of hearing or vision impairment were noted, and retinitis pigmentosa was excluded by careful ophthalmological investigations. Tests for cardiac functions, magnetic resonance imaging, electroencephalography, electromyography, and nerve-conduction studies showed no abnormalities. Biochemical investigation revealed an elevated lactic acid level in blood and cere-

brospinal fluid. A muscle biopsy revealed no morphological or histochemical abnormalities. The clinical findings are consonant with the spectrum of symptoms in Leigh disease (Tatuch et al. 1992). None of the members of the family shown in figure 1 showed evidence of a neurological disorder, on clinical examination.

DNA isolated from the muscle sample of the patient was analyzed for mutations in the mtDNA. Deletions and the point mutations at nucleotide positions 3243, 3271, 8344, and 8356 were excluded. In order to detect the NARP/Leigh mutations, a 633-bp DNA fragment containing nt 8993 was amplified and digested with *HpaII* for the T-to-G or the T-to-C mutation and with *AvaI* for the T-to-G mutation (de Vries et al. 1993). The PCR fragment could be digested with *AvaI* and *HpaII* (data not shown), indicating the T8993G transversion. A radioactive PCR was performed, and the signal intensities of the bands after *AvaI* digestion were compared, to determine the amount of mutated mtDNA. Radioactive [ $\alpha$ -<sup>32</sup>P]dCTP was added before the last elongation of the PCR reaction, to ensure that the intensity ratio of the *AvaI* fragments was a true reflection of the in vivo heteroplasmy. The patient's DNA from peripheral blood cells and muscle showed 74% and 79% mutated DNA, respectively. The same test was performed on DNA samples of the mother, sister, and maternal relatives of the patient. For the mother, peripheral white blood cells, fibroblasts, and a muscle sample were tested. However, even after prolonged autoradiographic exposure, no mutated mtDNA could be observed in any of the maternal tissues (data not shown). The sensitivity of the radioactive PCR test, estimated by dilution of the patient sample with a normal sample, was estimated to be >0.1%. Supportive evidence for the absence of the mutation in the mother can be derived from her healthy sibs and daughter, who were also negative for the T8993G mutation. To our knowledge, it has never been reported that the muscle tissue was negative in patients in whom a mtDNA mutation was found. The occurrence of a de novo mutation is the most likely explanation for our observations.

The spontaneous occurrence of mutated mtDNA in this patient is similar as the occurrence of deleted mtDNA seen in most patients with Pearson syndrome who also have a negative family history. Santorelli et al. (1993) and Tulinius et al. (1995) have also reported evidence for new mutations, but in these cases the asymptomatic mothers were carrier of appreciable amounts of mutated mtDNA. In cows, it has been observed that the mitochondrial genome can be replaced completely by a nucleotide-sequence variant within a single generation (Koehler et al. 1991). Very recently, it has been shown that low levels of deleted mtDNA do occur in oocytes, which are comparable to levels seen in tissue specimens of older subjects (Chen et al. 1995).



**Figure 1** Pedigree of the T8893G-positive patient with mental retardation and ataxia. The percentages given below the pedigree symbols are the percentages mutated mtDNA found in blood (bl), fibroblasts (fi), and muscle (mu).

Since point mutations do arise on aging, it is not unlikely that point mutations occur in oocyte mtDNA also. Attardi and coworkers have put forward the hypothesis that the mitochondrion is the unit of segregation (Yoneda et al. 1992). It might very well be that in our case the mutation has arisen before or during the mitochondrial expansion in the oocyte. In the stochastic purification following the dilution of the mtDNA down to one copy per organelle (Ashley et al. 1989), one mitochondrion with only mutated mtDNA may have survived. In the developing organism, the mutated mtDNA may have expanded because of the feedback mechanism of the functionally compromised mitochondria. One may wonder, then, whether this should be a rare event. Recent in vitro experiments with various mtDNA point mutations in mtDNA-less cells indicate that, in culture, the shift to either wild type or mutant may be dependent on the nuclear background (Yoneda et al. 1992; Chomyn et al. 1994; Mariotti et al. 1994; Dunbar et al. 1995). Obviously, identification of such nuclear-encoded, possibly tissue-specific, modifier genes will be needed to understand the genesis and frequency of mtDNA disorders.

I. F. M. DE COO,<sup>1,3</sup> H. J. M. SMEETS,<sup>1</sup>  
F. J. M. GABREËLS,<sup>2</sup> N. ARTS,<sup>1</sup> AND B. A. VAN OOST<sup>1</sup>  
Departments of <sup>1</sup>Human Genetics and <sup>2</sup>Neurology  
and Pediatrics, University Hospital, Nijmegen; and  
<sup>3</sup>Department of Neurology and Pediatrics, Academic  
Medical Centre, Amsterdam

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Address for correspondence and reprints: Dr. René F. M. de Coo, Department of Neurology and Pediatrics, Academic Medical Centre, Meibergdreef 9, NL-1105 AZ Amsterdam, The Netherlands.

Present address for B.A.v.O.: Department of Clinical Sciences of Companion Animals, Utrecht University, Utrecht. E-mail: b.vanoost@ukg.dgk.ruu.nl  
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### High Frequency of Mutations in Codon 98 of the Peripheral Myelin Protein Po Gene in 20 French CMT1 Patients

To the Editor:

Charcot-Marie-Tooth disease, characterized by distal muscle weakness and amyotrophy, decreased or absent tendon reflexes, and high arched feet (Dyck et al. 1993), is the most common inherited peripheral neuropathy, with a prevalence of 1 in 2,500 (Skre 1974). Two types of CMT have been distinguished on the basis of nerve conduction velocities (Lupski et al. 1991a). CMT type 1 is the most frequent, with markedly slowed velocities

(≤40 m/s) associated with hypertrophic onion bulb changes on nerve biopsy (Dyck et al. 1993).

Autosomal dominant CMT1 is genetically heterogeneous: CMT1A is caused by a 1.5-Mb duplication in 17p11.2 (Lupski et al. 1991b; Brice et al. 1992; Raeymaekers et al. 1992) and, more rarely, by a point mutation in the PMP22 (peripheral myelin protein, 22 kD) gene located in the duplicated region (Valentijn et al. 1992; Roa et al. 1993); CMT1B results from mutations in the Po (peripheral myelin protein zero) gene in 1q22-23. Forty-five percent (7/16) of the published mutations associated with CMT1 occur in exon 3 of Po (Hayasaka et al. 1993a, 1993b, 1993c; Himoro et al. 1993; Kulkens et al. 1993; Roa et al. 1993; Nelis et al. 1994; Latour et al. 1995).

In order to determine the cause of CMT1 in 20 unrelated patients without 17p11.2 duplications, mutations were sought in exon 3 of Po with three techniques: non-radioactive SSCP, automated sequencing, and PCR enzymatic restriction.

All index patients fulfilled the diagnostic criteria for CMT1, but family histories were absent in 10 cases. Median motor nerve conduction velocities (MMNCV) were ≤38 m/s ( $19.6 \pm 6.8$  m/s). Duplication in 17p11.2 was excluded with RFLP probes VAW409R3a (D17S122), EW401 (D17S61), or VAW412 (D17S125) (Raeymaekers et al. 1992).

The exon 3 of Po was amplified using intronic primers 1 (5'-GGGTCCTCTCACATGCTTCC-3') and 2 (5'-AACTGCTTCCCATAACCCTTG-3') (Pham-Dinh et al. 1993), which generate a 288-bp fragment. The PCR products were (1) analyzed by nonradioactive SSCP as described elsewhere (Orita et al. 1989; authors' unpublished data), and (2) directly sequenced using the *Taq* polymerase (Prism *Taq* DyeDeoxy Terminator Cycle Sequencing Kit, Perkin Elmer) and analyzed on an Applied Bio-Instruments automatic sequencer. The 5'→3' and 3'→5' strands were both analyzed using the primers 1 and 2, respectively. (3) The PCR products were then digested by *Cfo*I restriction enzyme (10 U/reaction) and analyzed on a 1% agarose gel.

Twenty unrelated CMT1 patients were first analyzed by nonisotopic SSCP for exon 3 of Po gene. An abnormal but different migration profile was detected for individuals SAL-297-5 and SAL-1187-3 (figs. 1 and 2). In both cases, a different new mutation involving codon 98 was found. In individual SAL-297-5, a missense Arg98Pro mutation was caused by a G→C transversion at nt 293, whereas a C→T transition at nt 292 was responsible for an Arg98Cys substitution in individual SAL-1187-3. Since these mutations abolished a *Cfo*I restriction site, which cleaves the 288-bp PCR fragment in two parts of 194 bp and 94 bp, it was possible to test relatives in both families for the presence of the restriction site. In family SAL-297, the restriction site was present in five